AL/OE-TR-1995-0009
VOLUME III of III

ARMSTRONG LABORATORY

GENETIC TOXICITY EVALUATION OF IODOTRIFLUOROMETHANE (CF_3I)

VOLUME III: RESULTS OF THE FORWARD MUTATION ASSAY USING L5178Y MOUSE LYMPHOMA CELLS

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January 1995

FINAL REPORT FOR THE PERIOD MARCH THROUGH DECEMBER 1994

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NOTICES

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

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VOLUME III

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Terry A. Child

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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13. ABSTRACT (Maximum 200 words)
Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated, used L5178Y mouse lymphoma cells from clone 3.7.2C to assess the capability of iodotrifluoromethane (CF_3I) to induce gene and chromosomal mutations at the thymidine kinase (*tk*) locus in the absence and presence of exogenous S9 metabolic activation.

To test this volatile material, cell cultures were placed in 15 ml round-bottom glass blood tubes sealed with serum stoppers, and, using a syringe, predetermined volumes of air were removed from each tube and nominal concentrations of CF_3I were introduced. Three tubes were used for each concentration, a tube containing 5 ml of cells in media without metabolic activation, a tube containing 5 ml of medium. After a four-hour exposure period at 37°C, the sham tubes were allowed to cool to room temperature, and the concentrations of the test material were measured using infrared (IF) analysis.

CF_3I was tested in two preliminary concentration range-finding assays and one mutagenesis assay, with each assay conducted in the absence and presence of metabolic activation. Contrary to information in the provided MSDS that CF_3I was insoluble in water, CF_3I appeared to be soluble in cell culture media because concentration-related increases in toxicity were obtained in each assay. In the mutagenesis assay, the negative control cloning efficiency and spontaneous mutation frequency met the criteria for acceptability, and positive control mutant frequencies were within the historical ranges for the laboratory, and when tested to the maximum concentration that cold be obtained, CF_3I was negative in the presence of toxicity (-). Therefore, CF_3I did not induce gene or chromosomal mutations in mammalian cells *in vitro*.

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PREFACE

The U.S. Air Force is investigating chemical replacements for the fire suppressant/extinguishant Halon 1301. Iodotrifluoromethane (CF_3I) is closely related structurally to Halon 1301 (CF_3Br) and may serve as a "drop in" extinguishant replacement. Results from laboratory animal *in vivo* studies indicate that CF_3I has a low order of acute toxicity. A comprehensive literature search indicated that no information was available on the mutagenic potential of. CF_3I. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of CF_3I. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substances Control Act) Health Effects Testing Guidelines.

This document, Volume III of III, serves as a final report detailing the results of the forward mutation assay using L5178Y mouse lymphoma cells. Volumes I and II describe, respectively, the results of the *salmonella typhimurium* histidine reversion assay (Ames assay) and the *in vivo* mouse bone marrow erythrocyte micronucleus testing.

The research described herein began in March 1994 and was completed in December 1994 by Genesys Research, Inc., Research Triangle Park, NC under a subcontract to ManTech Environmental Technology, Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F30). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

SUMMARY

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated used L5178Y mouse lymphoma cells from clone 3.7.2C to assess the capability of iodotrifluoromethane (CF_3I) to induce gene and chromosomal mutations at the thymidine kinase (*tk*) locus in the absence and presence of exogenous S9 metabolic activation.

To test this volatile material, cell cultures were placed in 15 ml round-bottom glass blood tubes sealed with serum stoppers, and, using a syringe, predetermined volumes of air were removed from each tube and nominal concentrations of CF_3I were introduced. Three tubes were used for each concentration, a tube containing 5 ml of cells in media without metabolic activation, a tube containing 5 ml of cells in media plus the metabolic activation mixture, and a "sham" tube containing only 5 ml of medium. After a four-hour exposure period at 37°C, the sham tubes were allowed to cool to room temperature, and the concentrations of the test material were measured using infrared (IR) analysis.

CF_3I was tested in two preliminary concentration range-finding assays and one mutagenesis assay, with each assay conducted in the absence and presence of metabolic activation. Contrary to information in the provided MSDS that CF_3I was insoluble in water, CF_3I appeared to be soluble in cell culture media because concentration-related increases in toxicity were obtained in each assay. In the mutagenesis assay, the negative control cloning efficiency and spontaneous mutation frequency met the criteria for acceptability, and positive control mutant frequencies were within the historical ranges for the laboratory, and when tested to the maximum concentration that could be obtained, CF_3I was negative in the presence of toxicity (-). Therefore, CF_3I did not induce gene or chromosomal mutations in mammalian cells *in vitro*.

GENESYS RESEARCH INCORPORATED'S GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

IN VITRO FORWARD MUTATION ASSAY OF IODOTRIFLUOROMETHANE (CF_3I) USING THE L5178Y/$tk^{+/-}$ MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY (MLA) WITH COLONY SIZING, WITH AND WITHOUT METABOLIC ACTIVATION

Genesys Research Incorporated's portion of the above titled study was reviewed for compliance with Quality Assurance (QA) regulations and with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The practices used in the study were found to be in compliance with these regulations.

Ann D. Mitchell 12/17/94

Ann D. Mitchell, Ph.D. Date
Study Director

GENESYS RESEARCH INCORPORATED'S QUALITY ASSURANCE STATEMENT

With the exception of the handling, storage, dilution (for exposure of the cells) and analytical chemistry of the test material, which were the responsibility of ManTech Environmental Technology, Incorporated, the data and the report for the following study carried out at Genesys Research, Incorporated has been reviewed and approved for compliance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The final report accurately describes the methods that were used and accurately reflects the raw data of the study.

ManTech Environmental Technology Incorporated Study Number: 1093-F30

Genesys Research, Incorporated Study Number: 94036

Type Study: L5178Y/$tk^{+/-}$ Mouse Lymphoma *In Vitro* Mammalian Cell Mutagenesis Assay

Protocol Signed by Study Director: March 19, 1994

Date Testing Started: March 30, 1994

Critical Phase Audit(s): April 20 and May 17, 1994

Date Testing Completed: May 20, 1994

Date Draft Report Audited: September 25 and October 1, 1994

Date Audit Findings Reported to Management: April 20, May 17 and October 1, 1994

Approved: Helen M. King
Helen M. King, B.S.
Quality Assurance Officer for Genesys

Date: 12/17/94

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of Iodotrifluoromethane

Project Number: 1093-F30

Study Director: Allen Ledbetter

ManTech Environmental Technology's portion of this study was conducted in accordance with EPA Good Laboratory Practice Regulations (GLP) as set forth in the Code of Federal Regulations (40 CFR 792). There were no significant deviations, in the work conducted by ManTech, from the aforementioned GLP regulations that would have affected the integrity of the study or the interpretation of the test results. The ManTech generated raw data have been reviewed by the Study Director, who certifies that the information contained in this report represents an appropriate and accurate conclusion within the context of the study design and evaluation criteria. Deviations are listed below:

1. The sponsor was responsible for the test substance characterization, stability and homogeneity analysis.

All original ManTech generated raw data are retained in the ManTech Environmental Technology's Archives, at 5 Triangle Drive, Research Triangle Park, NC 27709, with a copy of the final study report.

SUBMITTED BY:

Study Director:

Allen Ledbetter 12/16/94

Allen Ledbetter Date

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED
QUALITY ASSURANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of Iodotrifluoromethane

Project Number: 1093-F30

Study Director: Allen Ledbetter

Report Audit Dates:

This study has been subjected to inspections and the report has been audited by ManTech Environmental Technology's Quality Assurance Unit. The report describes the methods and procedures used in the study and the reported results accurately reflect ManTech's raw data. ManTech's raw data and a copy of the final report will be stored in room 210 in the MET building at Research Triangle Park, NC. The sponsor was responsible for the Iodotrifluoromethane characterization, stability and homogeneity analyses.

The following are the inspection dates, and the dates inspection reports were submitted:

Phase(s)	Date(s) of Inspection	Report Submitted to Study Director	Report Submitted to Management
Protocol	8/2/94	8/2/94	8/2/94
(Partial) Data Review	11/23/94		
Data Review	12/15/94	12/16/94	12/16/94

12/16/94

Terry F. Walser Date
Quality Assurance Officer

IN VITRO FORWARD MUTATION ASSAY OF IODOTRIFLUOROMETHANE (CF_3I) USING THE L5178Y/*tk*$^{+/-}$ MOUSE LYMPHOMA CELL MUTAGENESIS ASSAY (MLA) WITH COLONY SIZING, WITH AND WITHOUT METABOLIC ACTIVATION

1. INTRODUCTION

Under subcontract to ManTech Environmental Technology, Incorporated (ManTech), Dayton, Ohio (ManTech/Dayton) Genesys Research, Incorporated (Genesys) used L5178Y mouse lymphoma cells from clone 3.7.2C to assess the capability of iodotrifluoromethane (CF_3I) to induce gene and chromosomal mutations at the thymidine kinase (*tk*) locus in the absence and presence of exogenous S9 metabolic activation. Allen Ledbetter, ManTech Environmental Technology, Incorporated, Research Triangle Park, North Carolina (ManTech/RTP), was responsible for handling, storage, dilution (for exposure of the cell cultures), and analytical chemistry of the test material.

Testing at Genesys consisted of all procedures not performed by ManTech/RTP and was conducted under the direction of Ann D. Mitchell, Ph.D., Study Director, by J. Thom Deahl, M.S., and Diane M. Brecha, B.S., Genetic Toxicologists, in accordance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97). Testing was initiated with a preliminary concentration range-finding assay on March 30, 1994 and concluded on May 20, 1994 with mutant colony sizing for the mutagenesis assay of CF_3I. The protocol, a protocol amendment, raw data obtained by Genesys, and a copy of this report will be retained in Genesys' archives located at 2300 Englert Drive, Durham, NC 27713.

2. BACKGROUND

The development of the L5178Y mouse lymphoma cell mutagenesis assay (MLA) in the early 1970's by Clive and associates and further changes in the assay during the past two decades have been described by Clive *et al.* (in preparation).

The MLA detects mutations affecting the heterozygous thymidine kinase (*tk*) locus of L5178Y/*tk*$^{+/-}$-3.7.2C mouse lymphoma cells. In the MLA, two classes of mutants, both detected by their ability to form colonies in the presence of trifluorothymidine (TFT), which is toxic to *tk*$^{+/-}$ cells, can be distinguished based on colony size: large (λ) colony mutants which have normal 11b chromosomes, the site of the *tk* locus, and small (σ, slowly growing) colony mutants which often have cytogenetic damage to chromosome 11b that can be detected in conventionally-stained and banded chromosomes (Blazak *et al.*, 1986; Clive *et al.*, 1980; Hozier *et al.*, 1981, 1982; Moore *et al.*, 1985). Hence, chemicals that induce large numbers of σ colony mutants are generally considered to be clastogens

(chromosome-breaking chemicals, Moore *et al.*, 1985). This feature distinguishes the L5178Y mouse lymphoma assay from mammalian cell mutagenesis assays that measure effects at hemizygous loci (such as *hprt*, *aprt*$^{+/o}$, or *tk*$^{+/o}$), as the latter cannot detect slowly growing mutants (DeMarini *et al.* , 1989).

Because chemicals can induce a continuous spectrum of genetic damage, from alterations at the molecular level to extensive damage (including deletions, chromosomal breakage and rearrangements) before toxic levels are reached, it is necessary to maximize σ colony mutant recovery in the MLA in order to obtain complete information on this spectrum of genetic events.

3. METHODS

3.1. Identification, Storage, and Dilution of the Test Materials

The test material, iodotrifluoromethane (CF_3I; molecular weight 195.91; CAS Number 2314-97-8), a colorless gas, was received in steel gas cylinders from ManTech/Dayton on February 27, 1994 and on March 3, 1994 transferred to Allen Ledbetter, ManTech/RTP, who was responsible for handling, storage, and dilution of the test material. The CF_3I was stored at ManTech/RTP in the original containers at room temperature (approximately 72°F). ManTech/Dayton documented the strength, purity, and composition of the test material and provided a Material Safety and Data Sheet (MSDS) from Pacific Scientific for CF_3I. Upon acceptance of the final report, the remaining test material will be returned to the Sponsor. No reserve sample will be retained by ManTech/RTP.

3.2. Controls

The positive control chemicals were hycanthone (HYC, CAS No. 3105-97-3), a chemical that induces mutagenesis, with predominantly σ mutants, in the absence of an exogenous metabolic activation system, and cyclophosphamide (CP, CAS No. 50-18-0), a chemical that induces mutagenesis, with predominantly σ mutants, only with exogenous metabolic activation. The negative control was air. For assays using metabolic activation, the activation mixture was also added to the controls.

3.3. Metabolic Activation Preparations

Rat liver S9 homogenate, in KCl buffer, prepared from Aroclor 1254-induced male Sprague-Dawley rats, was obtained from Molecular Toxicology, Inc., Annapolis, Maryland and stored frozen in liquid nitrogen. It was thawed and used to prepare an S9 mixture immediately before the chemical exposure step of each assay. The final concentrations of the S9 mixture for the preliminary concentration range-finding assays included 2.4 mg/ml NADP and 4.5 mg/ml sodium isocitrate, which were prepared in serum-free medium, pH adjusted to 7.0 with 1N NaOH, and filter sterilized before adding 50 µl/ml S9 homogenate, then adding the mixture to the cells in F_{5HP} medium.

To reduce potential toxicity of the S9 mixture for the mutagenesis assay, the NADP concentration was reduced to 1.2 mg/ml and the S9 homogenate was reduced to 25 µl/ml; all other components were unchanged.

3.4. Cell Culture

L5178Y mouse lymphoma cells, clone 3.7.2C, provided by Dr. Donald Clive, Burroughs Wellcome Co., Research Triangle Park, NC, are stored in liquid nitrogen at Genesys. The cells were grown as a suspension culture in F_{10HP} medium (see composition below), cleansed of homozygous ($tk^{-/-}$) cells with medium containing 0.1 µg/ml methotrexate, as described by Mitchell *et al.*, 1988, and used as target cells for chemical exposure.

3.5. Media

L5178Y cells were cultivated in Fischer's medium for leukemic cells of mice supplemented with 31 µg/ml penicillin (1650 units/mg), 50 µg/ml streptomycin sulfate, 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate, 25 mM HEPES buffer, and 10% heat-inactivated horse serum to make F_{10HP}. F_{5HP}, containing 5%, rather than 10%, heat-inactivated horse serum, was the medium used for exposures in the presence of exogenous metabolic activation. F_{10P} was the medium used for exposures in the absence of exogenous metabolic activation and F_{10P} was used during the expression period. The horse serum concentration was 20% in incomplete cloning medium (ICM), which did not contain agar. BBL agar (0.22%, final concentration) was added to ICM to make complete cloning medium (CCM). The selective cloning medium contained TFT at a final concentration of 1 µg/ml.

3.6. Exposure of Cell Cultures

For testing this volatile material in the preliminary concentration range-finding and mutagenesis assays, three sterile 15 ml round-bottom glass blood tubes, sealed with red rubber serum stoppers, were prepared for each concentration level: a tube for the culture tested without activation, a tube for the culture tested with activation, and a sham tube that contained medium only (no cells or S9) which was used to estimate post-exposure infrared (IR) analysis of the concentration of test material in the other two tubes.

To provide a maximum available volume for the test material, each culture contained approximately 2.5 x 10^6 cells in 5 ml of F_{10HP} for cultures tested without exogenous metabolic activation, or in 1.5 ml S9 mix plus 3.5 ml F_{5HP} for cultures tested with metabolic activation. Therefore, at least 10 ml/tube was available for the volatile test material. After the cultures had been placed in the tubes at Genesys and the stoppers replaced, the tubes to be exposed to the CF_3I were transported to ManTech/RTP where, using a syringe, a predetermined volume of air was withdrawn from each tube and an equal volume of the pure test material was added. (This was not necessary for the negative control [air] or the positive controls; the latter were added directly to the cell cul-

tures before the tubes were sealed.) The three tubes per test material concentration were then returned to Genesys, and exposure was initiated by placing the tubes in a roller drum, and rotating them (~40 rpm) for 4 hours at 37°C.

After the exposure period, the cultures containing cells tested without and with S9 were transferred from the blood tubes to 15 ml plastic centrifuge tubes for subsequent steps in the assays. The sham tubes were allowed to cool to room temperature prior to analysis of the concentrations of the test materials by IR.

3.7. IR Calibration and Analysis

The IR instrument (Miran 1A, Foxboro Corp., Foxboro, MA; operated with wavelength = 9.7 microns, pathlength = 6.75 meters, absorbance = 0.25, slit = 1, and range = X1) was calibrated using a "closed-loop" method prior to analyzing the sham tube atmospheres. Calibration curves were prepared (using a TI-60 calculator, Texas Instruments, Lubbock, Texas) for concentration versus recorder chart lines using the least-squares method. Due to the wide concentration ranges of CF_3I in the tubes (10% - 100%, 1% = 10,000 ppm) in each assay, a single calibration curve was prepared for each assay, and the volume analyzed from each tube was varied based upon the expected tube concentration. The atmospheres were analyzed by withdrawing a volume of the atmosphere from the tube with a gas-tight syringe and injecting the sample into the IR instrument, which was in the "close-loop" configuration. The number of chart lines was entered into the calculator, and the corresponding concentration obtained. The concentration was then corrected for the injection volume.

3.8. Preliminary Range-Finding Assays

Two range-finding assays of CF_3I were conducted, with and without metabolic activation, to determine the most effective concentrations of CF_3I to use in the mutagenesis assay. A series of 8 nominal (calculated theoretical) concentrations of CF_3I, from 100,000 to 800,000 ppm, were used for initial preliminary cytotoxicity testing with and without metabolic activation, and 6 nominal concentrations of CF_3I, from 55,000 to 900,000 ppm, were used for the second preliminary assay. The procedures followed were the same as for the mutagenesis assay (described below) except that the cells were not cloned.

For each culture, growth of the cells in suspension (SG) was calculated each day by dividing the cell concentration at the end of that time period by the initial cell concentration. Total suspension growth (TSG) was calculated by multiplying day one SG by day two SG; relative suspension growth (RSG) was calculated by dividing TSG of each culture by the average TSG of the medium controls. The results from these experiments were then evaluated to select concentrations for mutagenesis testing.

3.9. Mutagenesis Assay

After exposure of the cultures as described above, the cells were centrifuged at low speed (~250 x *g*) for 5 minutes and the supernatant removed. The cells were rinsed at least twice by resuspension and centrifugation in F_{10HP} medium and then resuspended in 10 ml of F_{10HP} for growth during a two-day expression period.

During the expression period, the cell density was determined each day, and cells were diluted as necessary to maintain an optimum growth rate. On the second day of each assay, cultures were selected for cloning and 3 x 10^6 cells were removed from each of the test material, negative and positive control cultures to be cloned. An aliquot of 1000 cells was then obtained from each of these cultures by serial dilution and was cloned to determine cloning efficiency. After adding 1 µg/ml of TFT, the remainder of the 3 x 10^6 cells from each culture were cloned to determine mutant frequency. The soft-agar cloning medium was allowed to gel at room temperature for 15-20 minutes, then the dishes containing the cells were placed in a humidified CO_2 incubator and incubated at 37°C for 14 days.

Colonies in the mutant count and cloning efficiency dishes were counted, and the colonies in the mutant count plates were sized, as described below, using an Artek 982B semi-automatic colony counter with a high resolution video camera. For each culture, the absolute cloning efficiency (CE) was calculated by dividing the number of colonies in the cloning efficiency dishes by the number of cells cloned to measure cloning efficiency; relative cloning efficiency (RCE) was calculated by dividing the CE of each culture by the average CE of the negative (medium) controls, and relative total growth (RTG) was obtained by multiplying RSG by RCE. The mutation frequency (MF) for each culture was calculated by dividing the number of mutant colonies by the number of cells plated and multiplying by the reciprocal of the cloning efficiency. The average mutation frequency of the solvent control cultures was subtracted from that of each treated sample to express each result as an induced mutation frequency (IMF).

3.10. Colony Size Analysis

Mutant colony size distribution measurements were made using the semi-automatic stepping function of the Artek 982B counter. For test materials yielding a positive response, the small, large, and total mutation frequencies are reported for each treated sample. For test materials yielding a negative response, the small, large, and total mutation frequencies are reported for the positive and negative controls.

3.11. Raw Data Collection

All observations, raw data collected, and calculations were recorded onto standard forms which were bound together with the study protocol at the conclusion of testing.

3.12. Analysis and Interpretation of Results

a. Data Collected

Experimental data collected for each sample included growth in suspension, cloning efficiency, and mutant counts. From these data were calculated relative total growth (defined in Mitchell *et al.*, 1988), absolute cloning efficiency of the medium controls, and total mutation frequencies. Small and large colony mutation frequencies were obtained for each sample that was sized as described above. For acceptable experiments, the cloning efficiencies of the solvent controls should be at least 70%, the average spontaneous (negative control) mutation frequencies should be $\geq 50 \times 10^{-6}$, and the positive control mutation frequencies should be within the historical ranges for the laboratory.

b. Criteria for Interpretation

The results were evaluated according to the categories of responses utilized by the U.S. EPA Gene-Tox Workgroup (Mitchell *et al.*, in preparation), as follows:

++ Strong positive response with evidence of a dose-response and an induced mutation frequency of at least 100×10^{-6} (Δ_{100}) at a relative total growth (RTG) $\geq$ 20%.

\+ Positive response with evidence of a dose-response and an induced mutation frequency of at least 70×10^{-6} (Δ_{70}) at a RTG $\geq$ 10%.

\- Negative response for which toxicity is evidenced by a RTG of 10 - 20%, and the positive control mutation frequency demonstrates that there are no inherent problems with the assay.

= Negative response with no toxicity, and the positive control mutation frequency demonstrates that there are no inherent problems with the assay.

E Equivocal response in which positive and negative results are obtained in repeated experiments, and no reason is found to give greater weight to the positive or the negative result.

Not-testable. The test material can not be tested to sufficiently high concentration to obtain a conclusive result in the MLA because of limited solubility, acidic pH shifts, the test material's dissolving plastic, etc.

Hence, biological significance was considered in the evaluation of the results. The final interpretation of the results was the responsibility of the Study Director.

4. RESULTS AND DISCUSSION

Summaries of the concentrations tested, results obtained in preliminary concentration range-finding experiments, and the results obtained for testing CF_3I in the L5178Y/$tk^{+/-}$ mammalian cell mutagenesis assay are presented in Tables 1 - 4.

4.1. Concentrations Tested

Table 1 summarizes the initial nominal concentrations of CF_3I in the sets of tubes without and with activation for each assay, the IR determined concentrations from the corresponding sham tubes, and the percent of test material recovered after the exposure periods. As illustrated, 82 to 103% of the test material was recovered in the first preliminary assay, ~35 to 73% recovery was obtained in the second preliminary assay, and recovery was ~50 to 71% in the mutagenesis assay.

Differences in the nominal and IR determined concentrations could have arisen from: (a) lack of precision in providing the nominal concentrations; (b) leakage of the test materials from the tubes during the exposure period; or (c) absorption of the test materials by the cell culture medium. Lack of precision and leakage are considered to have been minimal because of the relatively small variability in percent recovery for most samples in each assay. Although the provided MSDSs stated that CF_3I is insoluble in water (and, hence, would be expected to be insoluble in culture medium), the depressions in relative suspension growth obtained for CF_3I in the second preliminary assay and the mutagenesis assay would suggest that CF_3I was, indeed, at least partially soluble in the culture medium.

4.2. CF_3I Results

As shown in Table 2, in the first preliminary concentration range-finding assay of CF_3I, relative suspension growth of the cultures appeared to be slightly depressed at the highest concentration tested, a nominal concentration of 800,000 ppm, but no concentration-related depression in RSG was observed for lower tested concentrations. Because this assay yielded insufficient information for selecting concentrations for mutagenesis testing, the concentration range-finding assay was repeated.

In the second concentration range-finding assay of CF_3I (Table 3), concentration related depressions in RSG were obtained in the absence and presence of metabolic activation, from 96.2% RSG at 55,000 ppm CF_3I to 28.4% RSG at 900,000 ppm CF_3I in the absence of activation, and from 70% RSG at 225,000 ppm CF_3I to 16.5% RSG at 900,000 ppm CF_3I in the presence of activation. Therefore, the mutagenesis assay was conducted over a similar range of concentrations.

In the mutagenesis assay of CF_3I (Table 4), the average absolute cloning efficiencies of the negative controls (air) were 127.3% in the absence of activation and 136.1% in the presence of metabolic activation (data not shown); the spontaneous mutation frequencies averaged 57×10^{-6} in the absence of activation and 62×10^{-6} in the presence

of activation. Therefore, both the cloning efficiencies and the spontaneous mutation frequencies met the criteria for acceptability. Positive control mutant frequencies were within the historical ranges for the laboratory. In the absence of activation, 10.0 μg hycanthone/ml yielded an induced mutant frequency (IMF) of 734 x 10^{-6} at 14.9% relative total growth (RTG), and, in the presence of activation, 2.0 μg cyclophosphamide/ml yielded an IMF of 834 x 10^{-6} at 10.6% RTG. For both positive controls, primarily small (σ) colony mutants were produced.

The depression in RSG observed in the second preliminary assay was essentially reproduced in the mutagenesis assay of CF_3I, and the highest concentration tested, 900,000 ppm nominal, yielded 22% RSG without activation and ~32% RSG with activation. RTG values, which included cloning efficiencies, were ~22.4% at the highest concentration without activation and ~28.3% with activation; thus, RTG values in the range of 10 to 20% were not achieved with the highest concentration of CF_3I that could be evaluated under the conditions of testing.

It may be noted that although the provided MSDS for CF_3I indicated that this test material is not soluble in water, apparently CF_3I is at least partially soluble in medium, as the concentration-related depressions in RSG that were observed would not be expected with an insoluble material.

As illustrated in Table 4, induced mutation frequencies of only 14 to 21 x 10^{-6} were obtained for the four highest tested concentrations in the absence of activation, and only 1 to 14 x 10^{-6} for the five highest tested concentrations in the presence of activation, and these increases were not concentration-related. Therefore, there was no evidence to suggest that induced mutation frequencies of at least 70 x 10^{-6} (required for evaluating the result as positive, +) would be obtained with greater toxicity, e.g., in the range of 10 to 20% RTG. For this reason, and considering biological significance, the results obtained for CF_3I in the L5178Y/$tk^{+/-}$mouse lymphoma cell mutagenesis assay in the absence and presence of activation are evaluated as negative (-). Therefore, when tested to the maximum concentrations that could be obtained under the conditions of testing, iodotrifluoromethane (CF_3I) did not induce gene or chromosomal mutations in mammalian cells *in vitro*.

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Table 1: **Comparison of Nominal and IR Determined Percent[a] Recovered Concentrations of Iodotrifluoromethane (CF_3I) in the Preliminary Concentration Range-Finding Assays and the L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mammalian Cell Mutagenesis Assay.**

Nominal Concentration[b] (ppm)	IR Determined Concentration[c] (ppm)	Percent Recovered[d]
First Preliminary Assay		
100,000	83,023	83.0
200,000	206,654	103.3
300,000	275,728	91.9
400,000	344,811	86.2
500,000	442,377	88.5
600,000	500,553	83.4
700,000	602,359	86.1
800,000	653,263	81.7
Second Preliminary Assay		
55,000	19,023	34.6
110,000	70,566	64.2
225,000	164,385	73.1
450,000	314,136	69.8
750,000	462,420	61.7
900,000	377,550	42.0
Mutagenesis Assay		
125,000	79,610	63.7
250,000	176,884	70.8
500,000	305,887	61.2
650,000	425,606	65.5
750,000	454,184	60.6
900,000	517,777	57.5
1,000,000	496,814	49.7

a The same concentrations were used for cultures tested in the absence and presence of metabolic activation.
b Initial concentrations in tubes containing cells in culture medium (without and with metabolic activation) and the sham tubes containing medium.
c Concentration in the sham tube after the exposure period.
d (Concentration in sham tube after exposure + initial concentration in sham tube and tubes containing cell cultures) x 100.

Table 2: Relative Suspension Growth of L5178Y Cell Cultures Exposed to Increasing Concentrations of Iodotrifluoromethane (CF_3I) in the Absence and Presence of Activation in the Initial Concentration Range-Finding Assay.

Chemical	+/- S9	Concentration*	RSG (%)
Air	-	N/A	86.71
Air	-	N/A	113.29
CF3I	-	100,000 ppm	71.42
CF3I	-	200,000 ppm	83.66
CF3I	-	300,000 ppm	59.90
CF3I	-	400,000 ppm	59.18
CF3I	-	500,000 ppm	84.91
CF3I	-	600,000 ppm	74.29
CF3I	-	700,000 ppm	80.35
CF3I	-	800,000 ppm	49.08
Air	+	N/A	97.03
Air	+	N/A	102.97
CF3I	+	100,000 ppm	113.08
CF3I	+	200,000 ppm	103.04
CF3I	+	300,000 ppm	102.97
CF3I	+	400,000 ppm	91.42
CF3I	+	500,000 ppm	94.93
CF3I	+	600,000 ppm	117.79
CF3I	+	700,000 ppm	104.26
CF3I	+	800,000 ppm	77.00

* = Nominal Concentration; RSG(%) = Percent relative suspension growth.

Table 3: Relative Suspension Growth of L5178Y Cell Cultures Exposed to Increasing Concentrations of Iodotrifluoromethane (CF_3I) in the Absence and Presence of Activation in the Second Concentration Range-Finding Assay.

Chemical	+/- S9	Concentration*	RSG (%)
Air	-	N/A	106.53
Air	-	N/A	93.47
CF3I	-	55,000ppm	96.18
CF3I	-	110,000 ppm	83.61
CF3I	-	225,000 ppm	70.46
CF3I	-	450,000 ppm	40.96
CF3I	-	750,000 ppm	33.51
CF3I	-	900,000 ppm	28.39
Hycanthone	-	7.50 μg/ml	34.63
Hycanthone	-	10.00 μg/ml	29.70
Air	+	N/A	101.09
Air	+	N/A	98.91
CF3I	+	55,000 ppm	69.05
CF3I	+	110,000 ppm	67.77
CF3I	+	225,000 ppm	69.78
CF3I	+	450,000 ppm	69.16
CF3I	+	750,000 ppm	42.97
CF3I	+	900,000 ppm	16.48
Cyclophosphamide	+	1.25 μg/ml	72.63
Cyclophosphamide	+	2.50 μg/ml	78.19

* = NominalConcentration; RSG(%) = Percent relative suspension growth.

Table 4: **Results from the L5178Y/*tk*$^{+/-}$ Mouse Lymphoma Mammalian Cell Mutagenesis Assay of Iodotrifluoromethane (CF_3I) in the Absence and Presence of Metabolic Activation.**

Chemical	+/- S9	Conc.*	RSG(%)	RTG (%)	MF x 10^{-6}	IMF x 10^{-6}	Notes
Air	-	N/A	110.81	114.39	54		
Air	-	N/A	89.19	86.31	60		
CF3I	-	125,000 ppm	79.74	80.46	58	1	
CF3I	-	250,000 ppm	71.47	79.93	55	-	
CF3I	-	500,000 ppm	57.91	55.42	51	-	
CF3I	-	650,000 ppm	55.80	49.20	76	19	
CF3I	-	750,000 ppm	40.74	42.42	71	14	
CF3I	-	900,000 ppm	23.19	22.61	75	18	
CF3I	-	1,000,000 ppm	22.09	22.41	78	21	
Hycanthone	-	7.50 µg/ml	34.10	21.15	448	390	◆◆
Hycanthone	-	10.00 µg/ml	31.68	14.87	791	734	◆◆
Air	+	N/A	93.52	102.84	54		
Air	+	N/A	106.48	95.86	70		
CF3I	+	125,000 ppm	90.91	96.77	60	-	
CF3I	+	250,000 ppm	106.64	-**	-**	-**	
CF3I	+	500,000 ppm	76.41	81.60	63	1	
CF3I	+	650,000 ppm	73.32	64.90	76	14	
CF3I	+	750,000 ppm	54.62	60.34	74	12	
CF3I	+	900,000 ppm	32.29	37.67	73	11	
CF3I	+	1,000,000 ppm	31.88	28.28	67	5	
Cyclophosphamide	+	1.00 µg/ml	81.02	50.12	374	312	◆◆
Cyclophosphamide	+	2.00 µg/ml	20.31	10.63	896	834	◆◆
Cyclophosphamide	+	3.00 µg/ml	44.93	2.56	2,303	2,241	◆◆

* = Nominal Concentration; RSG(%) = Percent relative suspension growth; RTG(%) = Percent relative total growth; MF = mutant frequency; IMF = induced mutant frequency; ◆◆ = IMF $\geq 100 \times 10^{-6}$; **Not cloned for mutagenesis.

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